

AMENDMENTS TO THE SPECIFICATION

In accordance with the Examiner's suggestion, Applicants have amended pages 3, 7, 9, 12, 15 and 16 of the description to overcome the Examiner's objection to the Specification. Specifically, the following typographical errors have been corrected: "Shiwanella" has been changed to "Shewanella" and "Flexinobactor" has been changed to "Flexibacter." It is respectfully submitted that no new matter is presented by these amendments.

Applicants have also corrected the misspelling of "florescens" or "florescence" to "fluorescens" throughout the application, including the Listing of Claims.

[Page 3, last paragraph]:

In accordance with one aspect of the instant invention, there is also provided a composition where killed bacteria selected from a group consisting of *Shiwanella* *Shewanella* *putrefaciens*, *Pseudomonas* *fluorescens* *fluorescens*, *Vibrio alginolyticus* and *Flexinebacter* *Flexibacter* *columnaris* or their respective antigens are included along with recombinant AHMA and FP.

[Page 7, first paragraph under "Detailed Description"]:

One embodiment of the instant invention provides an oral vaccine comprising at least one recombinant protein AHMA. In one preferred embodiment of the invention, the composition comprises two recombinant proteins namely, recombinant AHMA and recombinant FP dissolved in an

emulsion. The cloning and expression of recombinant AHMA has been fully described in the US Patent Application No. 10/220,986 to Sin *et al.* filed on 7th March, 2001 entitled "Therapeutic and Prophylactic Agents Derived from *Aeromonas hydrophila* Bacterial Surface Proteins", the contents of which in its entirety are incorporated by reference herein. Cloning and expression of recombinant FP has been fully described in US Patent Application No. 09/196,161 to Sin *et al.* filed on 20th November, 1998 entitled "Recombinant Vaccine Against Infectious Diseases in Fish", the contents of which in its entirety are incorporated by reference herein. Other embodiments may incorporate other recombinant coat proteins from pathogens to enhance the protection of the vaccine against a wider spectrum of infections. Thus, an embodiment of the invention comprises recombinant proteins AHMA and FP along with killed bacteria selected from the group consisting of *Shewanella* *Shewanella putrefaciens*, *Pseudomonas fluorescens* *fluorescens*, *Vibrio alginolyticus* and *Flexinebacter* *Flexibacter columnaris*. Another embodiment of the invention includes besides the recombinant proteins and killed bacteria mentioned above, inactivated viruses from the group consisting of guppy reovirus and guppy nervous necrosis virus.

[Page 9, third paragraph]:

While recombinant AHMA may generate antibodies that cross-protect against other bacterial species, such as of *Aeromonas*, *Vibrio* and *Edwarsiella*, it may be desirable to add other bacterial antigens to the oral vaccine. Thus in one

embodiment of the invention, four killed bacteria namely, *Shewanella* *Shewanella putrefaciens*, *Pseudomonas fluorescens* *fluorescens*, *Vibrio alginolyticus* and *Flexineobacter* *Flexibacter* *columnaris* are incorporated. These bacterial infections are common in fish. However, caution must be exercised while selecting bacterial antigens for incorporation namely, that they must not cross-react with AHMA antibodies, as recombinant AHMA is one component of the oral vaccine. These selected bacteria may be inactivated by any method known to those skilled in the art, including irradiation, heat-inactivation or chemical treatment. Their antigenic proteins may also be made by recombinant methods for incorporation into the multicomponent oral vaccine.

[Pages 11-12, split paragraph from bottom of page 11 to top of page 12]:

The dosage of component of the vaccine is made in accordance with the body weight of the intended subject so as to provide an immunologically sufficient amount to elicit protective response. Thus dosage of AHMA in the vaccine may range between 7 and 150 $\mu\text{g}/\text{gm}$ body weight. A more preferable amount would be 15-20 $\mu\text{g}/\text{gm}$, a most preferred amount would be about 17 $\mu\text{g}/\text{gm}$ body weight. Similarly the components may also be employed at immunologically effective dosage. Thus, an immunologically effective amount of recombinant FP may range between 7 and 150 $\mu\text{g}/\text{gm}$ body weight, a more preferable dose range may be 15 and 20 $\mu\text{g}/\text{gm}$ and the most preferable dose may be 17 $\mu\text{g}/\text{gm}$ body weight of the immunized subject. Preferred amounts of inactivated virus or equivalent amounts of viral proteins for guppy reovirus and

guppy nervous necrosis virus may range between 10^3 to 10^6 viral particles per unit dose of the vaccine. The most preferred amounts to elicit a protective response may be 10^5 viral particles of each virus per dose of the vaccine. Similarly, killed bacteria or bacterial protein components to be used in the vaccine including *S. putrefaciens*, *P. fluorescens*, *V. alginolyticus* and *F. columnaris* may have a range of 2.5×10^5 to 2.5×10^7 cfu of each of the bacterium. The most preferred amount may be 2.5×10^6 cfu of each bacterium or its equivalent coat protein per unit dose of the vaccine.

[Page 15, first paragraph under “Example IV”]:

Four strains of bacteria (*Shewanella* *Shewanella* *putrefaciens*, *Pseudomonas* *fluorescens* *fluorescens*, *Vibrio* *alginolyticus* and *Flexibacter* *Flexibacter* *columnaris*) were grown separately. *S. putrefaciens*, *P. fluorescens* *fluorescens* and *V. alginolyticus* were cultured in TSB while the *F. columnaris* was cultured in Ordal culture media (0.2% tryptone, 0.05% yeast, 0.3% gelatin).

[Page 16, paragraph under “Example V”]:

The various embodiments of the oral vaccine were prepared employing the following dosages for every batch of 100 fish:

- (a) 0.7 mg AHMA for the oral vaccine comprising recombinant AHMA alone,
- (b) 0.7 mg AHMA+0.7 mg recombinant FP for the rAHMA-FP vaccine, (c) 0.7 mg AHMA+0.7 mg recombinant FP and 2.5×10^6 cfu of each or all the four bacteria, *Flexibacter* *columnaris*, *Pseudomonas* *fluorescens* *fluorescens*, *Shewanella*

Shewanella putrefaciens and *Vibrio alginolyticus* for the oral vaccine that also included bacterial components and (d) 0.7 mg AHMA+0.7 mg recombinant FP, 2.5×10^6 cfu of each or all the four bacteria, *Flexibacter columnaris*, *Pseudomonas fluorescens fluorescens*, *Shewanella Shewanella putrefaciens* and *Vibrio alginolyticus* and 10^5 viral particles of GPV and GNNV for the vaccine having viral antigens in addition. For the multi-component vaccine having AHMA, FP, the bacterial and viral antigens, all the 8 components were mixed in a total volume of 0.25 ml water and 0.5 ml palm oil. The mixture was vigorously stirred till it emulsified before being folded into 0.5 g of powered commercial eel feed. The other embodiments were also prepared in a similar manner using the respective amounts of antigen indicated.